Structure and polymorphism of 18-carbon fatty acyl triacylglycerols: effect of unsaturation and substitution in the 2-position[1]

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Abstract The polymorphic behavior of symmetric diacid triacylglycerols (TGs), 1,3-dioleoyl-2-stearoyl (OSO), 2-elaidoyl (OEO), and 2-vaccinoyl (OVO) glycerols were studied by differential scanning colorimetry (DSC) and X-ray diffraction and compared with the corresponding monoacid TGs triolein (OOO), tristearin (SSS), trielaidin (EEE), and trivaccinin (VVV). The monoacid TGs formed a bilayered structure in all the polymorphic forms. On quenching from the melt, the diacid TGs OEO and OVO formed a bilayered (D = 45 Å) β'-phase with the exception of OSO, which formed a hexagonally packed bilayered (D = 52 Å) α-phase. At −7°C, the α-phase of OSO quickly transformed to a bilayered (D = 45 Å) β'-phase. Incubation at the β'-phase melting temperature transformed OVO, OEO, and OSO into a trilayered (D = 65 Å) β-phase, where the 1,3-dioleoyl chains are segregated from the vaccinoyl, elaidoyl, or stearoyl chains into alternating layers. In summary, when all the acyl chains in a TG are the same (saturated, *cis* or *trans* unsaturated), the stable β-phase packs into a bilayered structure. However, when the 1- and 3-acyl chains are *cis* unsaturated (bent) and the 2-acyl chain is either saturated or *trans*-unsaturated (straight), a bilayered β'-phase can form, but transforms to a stable trilayered β-phase, where the 2-acyl chains form a layer between two different layers of 1,3-oleoyl chains.—**Kodali, D. R., D. Atkinson, T. G. Redgrave, and D. M. Small.** Structure and polymorphism of 18-carbon fatty acyl triacylglycerols: effect of unsaturation and substitution in the 2-position. *J. Lipid Res.* 1987. **28:** 403–413.

Supplementary key words differential scanning colorimetry • X-ray diffraction • phase behaviors • bilayer and trilayer structures • membranes

A specific molecular structure of TGs has important implications for their physical properties (1) and also perhaps some physiological properties such as enzymatic hydrolysis and subsequent metabolism (2, 3).

The most intriguing feature of these compounds is their ability to pack into two or more crystalline forms with similar lattice energy. X-ray diffraction and infrared absorption have been used to determine the thermal behavior. Three distinct polymorphic forms, α, β', and β, differing from each other in the hydrocarbon chain packing, have been characterized. This polymorphic phenomenon of TGs has been extensively studied by different workers (1, 4–7). Recently, DeJong and Van Soest (8) published a comprehensive packing analysis of mixed chain saturated TGs in the β-phase. However, the precise structure–phase behavior correlations are not well understood. The main factors that contribute to different modes of packing in each phase are the geometry of the glycerol backbone region and the packing of the long acyl chains along their short and long axes. These two factors are influenced by the acyl chain length and position of substitution on the glycerol backbone (1). The chain packing is further complicated by the presence of one or more double bonds.

In our first study we examined the influence of 3-acyl chain length on the polymorphic behavior of 1,2-dipalmitoyl-3-acyl-*sn*-glycerols (9). In another study we reported the three- and six-layer packing arrangement of 1,2-dioleoyl-3-acyl-*sn*-glycerols (10).

The present study examines the packing behavior of monoacid TGs, tristearin, triolein, trielaidin, and trivaccinin, and then the symmetric 1-2-dioleoyl-2-substituted TGs where the acyl chain esterified at the 2-position was systematically varied with stearoyl, elaidoyl, and vaccinoyl chains. The effects of unsaturation and position of substitution of the acyl chains on the phase behavior of these compounds were studied. Some of the polymorphic properties of monoacid TGs SSS, OOO, EEE, and VVV were reported in the literature (4, 11–15). The data for SSS is extensive and in this study we used data from references 4 and 11.

Abbreviations: TG, triacylglycerol; SSS, tristearin (C18:0); OOO, triolein (C18:1 *cis*-9); EEE, trielaidin (C18:1 *trans*-9); VVV, trivaccinin (C18:1 *trans*-11); OSO, 1,3-dioleoyl-2-stearoyl glycerol; OEO, 1,3-dioleoyl-2-elaidoyl glycerol; OVO, 1,3-dioleoyl-2-vaccinoyl glycerol; DSC, differential scanning colorimetry; TLC, thin-layer chromatography.

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MATERIALS AND METHODS

The fatty acids, vaccenic acid (C18:1, *trans*-11) elaidic acid (C18:1, *trans*-9), oleic acid, and stearic acid and the TGs triolein (OOO) and trielaidin (EEE) were purchased from Nu-Chek-Prep, Inc. (Elysian, MN). The purity of all the compounds used in this study was checked by TLC and found to be > 99% pure. The solvents used were HPLC grade from Fisher Scientific Co. (Medford, MA). 4-Dimethylaminopyridine and 1,1'-dicyclohexylcardodiimide were purchased from Aldrich Chemical Co. (Milwaukee, WI).

Syntheses

Trivaccinoyl (C18:1, *trans*-11) glycerol was synthesized by the esterification of 3 mol of vaccinic acid with 1 mol of glycerol by using 1,1'-dicyclohexylcarbodiimide in the presence of 4-dimethylaminopyridine. The syntheses of OSO, OEO, and OVO were accomplished by the synthetic procedures described below.

Preparation of 1,3-dihydroxypropane-2-one 1,3-dioleate

To a solution of 0.9 g of 1,3-dihydroxyacetone (10 mmol) in carbon tetrachloride (20 ml) oleic acid (5.93 g; 21 mmol) and 4-dimethylaminopyridine (2.44 g; 20 mmol) were added. To this reaction mixture 4.33 g of 1,1-dicyclohexylcarbodiimide (21 mmol) in carbon tetrachloride (10 ml) was added in small portions (in ~10 min) with stirring at room temperature. As judged by TLC in hexane–ethyl acetate 4:1 the reaction was complete in 2.5 hr. At the end of the reaction, the precipitated dicyclohexylurea was removed by filtration. The solvents were rotoevaporated and the residue was dissolved in a minimum amount of hexane (~25 ml) and kept at 0°C for a few hours. Crystallized 4-dimethylaminopyridine was removed and the filtrate was concentrated. The residue was recrystallized from an ethyl acetate (18 ml), methanol (10 ml), and water (0.5 ml) mixture to give 1,3-dihydroxypropane-2-one 1,3-dioleate, yield 5 g (81%) mp 43°C (lit. 43–44°C) (16).

1,3-Dioleoylglycerol

1,3-Dioleoylglycerol was synthesized according to the literature procedure (16). The reaction was complete in 15 min and the product was crystallized from pentane, yield 82% mp 25.5°C (lit. mp 20–22°C). The purity of the compound was checked on boric acid-impregnated TLC plates developed on chloroform–acetone 90:10 with 1,2-diacylglycerol as a reference. On this system there was no detectable isomeric 1,2-dioleoylglycerol.

1,3-Dioleoyl, 2-substituted glycerols

The 2-substituted 1,3-dioleoylglycerols were prepared by the condensation of 1,3-dioleoylglycerol with an appropriate fatty acid. A typical procedure for the synthesis of 1,3-dioleoyl-2-stearoyl glycerol is given below.

To a solution of 621 mg of 1,3-dioleoylglycerol (1 mmol) in carbon tetrachloride (10 ml), 313 mg of stearic acid (1.1 mmol) and 122 mg of 4-dimethylaminopyridine (1 mmol) were added. 1,1'-Dicyclohexylcarbodiimide (227 mg; 1.1 mmol) was added to the above reaction mixture with stirring at room temperature. After the reaction was complete (2–3 hr; as judged by TLC, solvent system hexane–isopropyl ether 70:30), the precipitated dicyclohexylurea was filtered off and the filtrate was concentrated. The product was purified by column chromatography under pressure, eluted with a gradient of hexane to hexane–isopropyl ether 90:10. The yield was 743 mg (84%), mp 25.8°C.

OEO and OVO were prepared similarly by the condensation of 1,3-dioleoylglycerol with elaidic and vaccinic acids, respectively, in 80–85% yield.

At all stages of synthesis the intermediates and the final products were identified by ^{13}C NMR. OSO, OEO, and OVO showed two peaks in the carbonyl region. The carbon chemical shift of 1,3-dioleoyl carbonyl was observed between 173.11 and 173.23 ppm and the carbonyl carbon of the acyl chain esterified to the glycerol secondary hydroxyl appeared between 172.75 to 172.86 ppm. Previously it had been shown (10) that when the fatty acyl chains substituted on glycerol carbon one and three were chemically different, the carbonyl carbon signal between 173.11 and 173.23 was split into a double peak corresponding to the two different acyl groups. The spectra of OVO, OSO, and OEO showed a single peak in this region, which confirmed that both the primary hydroxyls were esterified with the same fatty acid.

Differential scanning calorimetry (DSC)

DSC measurements were performed with a Perkin-Elmer DSC-2 instrument over the temperature range of −50°C to 60°C at a cooling and heating rate of 5°C/min. The samples of pure lipids (1.5 to 3.5 mg) were placed in DSC stainless-steel pans. Transition temperatures are given as peak temperatures. The samples (from solvent of crystallization) that were solid at room temperature (EEE, VVV, OSO) were loaded into the DSC apparatus without melting and the temperature was lowered to at least 30° below the melting temperature of each compound and then heated to melt. The samples that were liquids at room temperature (OEO, OVO) were also loaded into DSC at room temperature but cooled to −53°C and then heated to an appropriate temperature (0°C for OEO; −10°C for OVO) and incubated at that temperature for various lengths of time to obtain the stable polymorph before heating to melt. After melting the stable polymorphic form, the subsequent cooling and heating runs were done without any lapse of time. The baselines were drawn connecting the flat tails of the beginning and the end of the peak. Transition enthalpies were determined from the area under the peak measured with a planimeter and calibrated with a gallium standard.

X-ray diffraction

X-ray diffraction patterns were recorded using either photographic film or position-sensitive proportional detector methods. For film recording, nickel-filtered Cukα radiation (λ = 1.5418 Å) from an Elliot GX-6 rotating anode X-ray generator (Elliot Automation, Borehamwood, England) was collimated by double-mirror optics (17).

For counter recording, CuKα radiation from a microfocus X-ray generator (Jarrel-Ash, Waltham, MA) was line-focussed (100 μm × 14 mm) by a single mirror and further collimated using the slit optical system of a Luzzati-Baro camera. Data were recorded using a linear position-sensitive detector (Tennelec, Oak Ridge, TN) and associated analysis system (Tracor Northern, Middleton, WI).

In all cases samples were contained in thin-walled quartz capillary tubes (Charles Supper Co., Inc., Natick, MA; internal diameter 1.0 mm) and mounted in a variable temperature sample holder with a temperature stability of ± 1°C.

The film recording was used to identify and compare the short and long spacings of the most stable phase (β-phase) of all the compounds with the values obtained for the same phase of the compounds by using the linear position-sensitive detector recordings. The identifications of all the phases at different temperatures of all the compounds were done by using a linear position-sensitive detector.

RESULTS

The polymorphic behavior of OOO was reinvestigated. The data for OOO and the other monoacid TGs are given in **Table 1**. The polymorphic behavior of EEE, VVV, OSO, OEO, and OVO was studied extensively. The thermal behavior of these compounds (EEE, VVV, OSO, OEO, OVO) on heating and cooling at 5°/min is shown in Figs. 1a–5a and the X-ray diffraction patterns are shown in Figs. 1b–5b. The temperatures at which the X-ray diffraction experiments were recorded are indicated by arrows on the thermal scans (Figs. 1a–5a) and are numbered to identify the corresponding X-ray diffraction patterns in Figs. 1b–5b.

EEE

The sample from solvent of crystallization melted to an isotropic liquid at 42°C with a transition enthalpy of 36.0 Kcal/mol as shown in the first heating scan at the top of **Fig. 1a**. The X-ray diffraction pattern of the phase from solvent of crystallization showed (Fig. 1b1) a strong diffraction corresponding to 4.5 Å indicating a β-phase with 45 Å long spacing. On cooling from the isotropic liquid, the beginning of crystallization occurred at 11°C (peak at 9°C) with an enthalpy of formation of 19.5 Kcal/mol (Fig. 1a). The X-ray diffraction experiment for the corresponding phase was obtained by quenching the isotropic liquid in ethanol–dry ice mixture and holding the temperature in the sample holder at −9°C. The X-ray diffraction pattern thus obtained is shown in Fig. 1b2. A single strong diffraction corresponding to 4.07 Å characteristic of α-phase was present. The long spacings for this α-phase indexed to a bilayer of 50.5 Å thickness. The DSC reheating scan (Fig. 1a) showed the beginning of melting of the α-phase at 14°C (peak at 15°C) with an enthalpy of 4.5 Kcal/mol. The α-phase melting was eclipsed by an immediate recrystallization with a peak transition temperature of 17°C and with an enthalpy of 21.5 Kcal/mol. This crystallization continued up to 36°C where the final endotherm started to appear. The peak transition temperature of the final endotherm was 41°C with the transition enthalpy of 30.9 Kcal/mol. When the X-ray diffraction sample temperature was increased at

TABLE 1. Physical characteristics of the α, β', and β phases of the monoacid triglycerides

Triglyceride[a]	Melting Point α	Melting Point β'	Melting Point β	T_c[b]	Long Spacings α	Long Spacings β'	Long Spacings β	ΔH of β→ Melt	Reference
		°C		°C		Å		*Kcal/mol*	
Tristearin SSS	54.7	63.2	73.5		50.6	47	45.1		4
Tristearin SSS	55	61;64	73					45.7	11
Trielaidin EEE	15.5	37(?)	42						12
Trielaidin EEE	15		41					34.92	11
Trielaidin EEE	16.6		42		48		45		15
Trielaidin EEE	15		42	11	49.6	[c]	45	36.0	this study
Triolein OOO	−32	−12	4.9						12
Triolein OOO	−37	−12; −8; −5	5					22.8	11
Triolein OOO	−32	−13	5.5		45.2	45.8	43.3		13
Triolein OOO			5			45	42.5		this study
Trivaccinin VVV	15	23	43					34.2	14
Trivaccinin VVV			42	13	52	[c]	45	36.2	this study

[a] See also ref. 1.
[b] Temperatures of onset of crystallization.
[c] Absent.

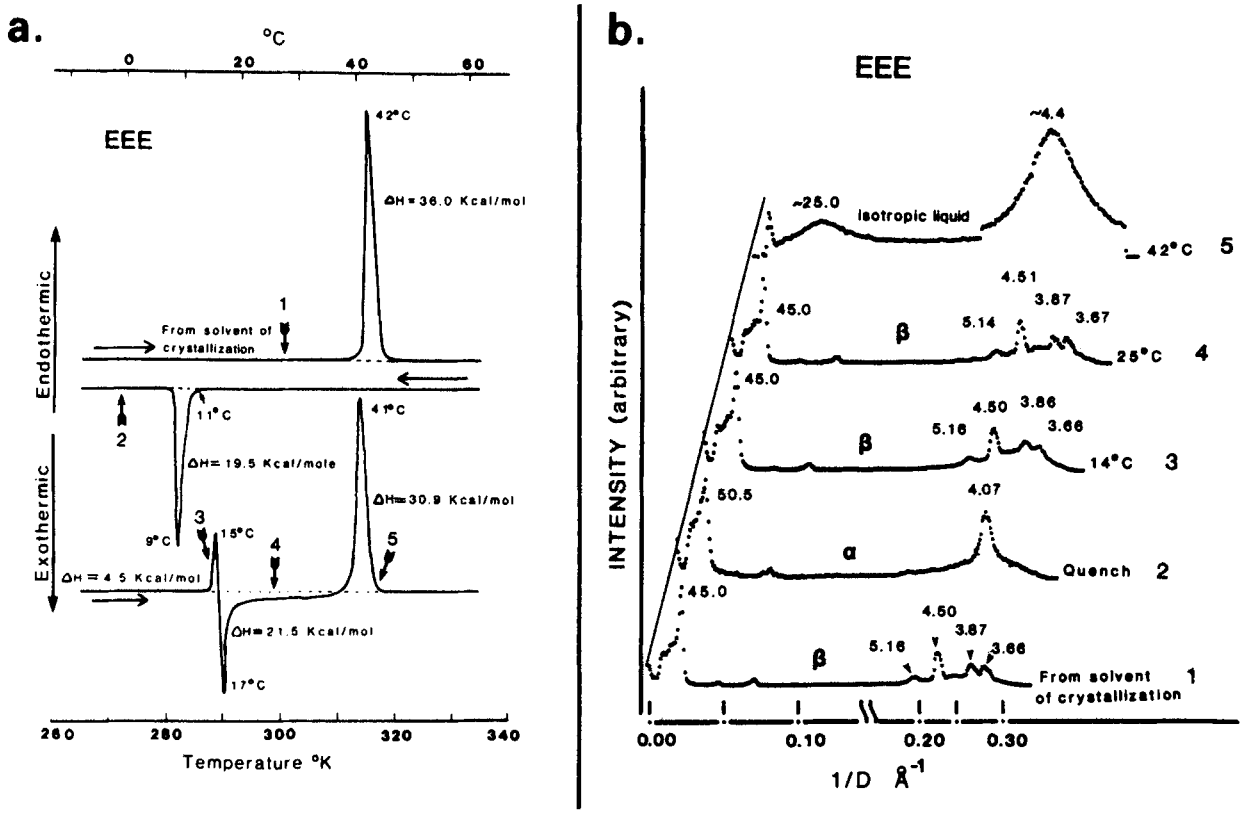

Fig. 1. a: Differential scanning calorimetry of trielaidoylglycerol. The numbered arrows indicated the temperatures at which the X-ray diffraction experiments were performed. Heating →; cooling ←. b: X-ray diffraction patterns of trielaidoylglycerol at different temperatures. The numbers on the righthand side of each diffraction pattern indicate the corresponding temperatures on DSC scans (numbered arrows) for samples with similar thermal history. The polymorphic form identified from wide-angle diffraction lines is given on top of each diffraction pattern. The D values in Å units are given at the top of the peaks.

a rate of ~0.3°C/min up to 14°C, the 4.1 Å short spacing completely disappeared and a characteristic strong β-phase diffraction (D spacing 4.5 Å) was observed (Fig. 1b3). The X-ray diffraction pattern at 25°C (Fig. 1b4) was similar to the X-ray diffraction pattern at 14°C and these two diffraction patterns were, in turn, identical to the diffraction pattern of the β-phase obtained from the solvent of crystallization (Fig. 1b1). The diffraction pattern of the β-phase was evident until 41°C, where it slowly transformed to the diffuse scattering typical of an isotropic liquid. The diffraction pattern obtained at 42°C for the isotropic liquid is shown in Fig. 1b5.

VVV

The phase obtained from solvent of crystallization melted to an isotropic liquid at 42°C with an enthalpy of 36.2 Kcal/mol. The X-ray diffraction pattern of this phase (**Fig. 2b1**) showed multiple short spacings with the strong D spacing of 4.5 Å characteristic of β-phase and a long spacing of 45 Å. On cooling the isotropic liquid, the beginning of crystallization occurred at 13°C (peak at 8°C) with an enthalpy of formation of 19.2 Kcal/mol. The X-ray diffraction pattern for the corresponding phase, obtained after quenching the isotropic liquid in dry ice–ethanol and maintaining the sample temperature at −10°C, showed a single strong diffraction corresponding to 4.13 Å characteristic of an α-phase with a periodicity along the long axis of 52 Å (Fig. 2b2). The DSC reheating scan showed that the α-phase recrystallized without melting at 17–22°C and an enthalpy of 14.7 Kcal/mol. This crystallization continued up to the final endotherm, indicating melting to an isotropic liquid at 41°C with an enthalpy of 31.7 Kcal/mol. On raising the X-ray sample temperature, the 4.13 Å short spacing was observed until 14°C, where the strong diffraction of the β-phase appeared (D = 4.46 Å). The β-phase diffraction pattern remained (Fig. 2b3 and 2b4) until 40–41°C where the intensity of the diffraction peaks decreased and was replaced by a diffraction characteristic of an isotropic liquid (Fig. 2b5).

OSO

The form obtained from solvent of crystallization melted to an isotropic liquid at 25°C with a transition enthalpy of 31.8 Kcal/mol. The X-ray diffraction pattern of this phase is characteristic of a stable β-phase with a strong diffraction corresponding to 4.52 Å and a long spacing periodic-

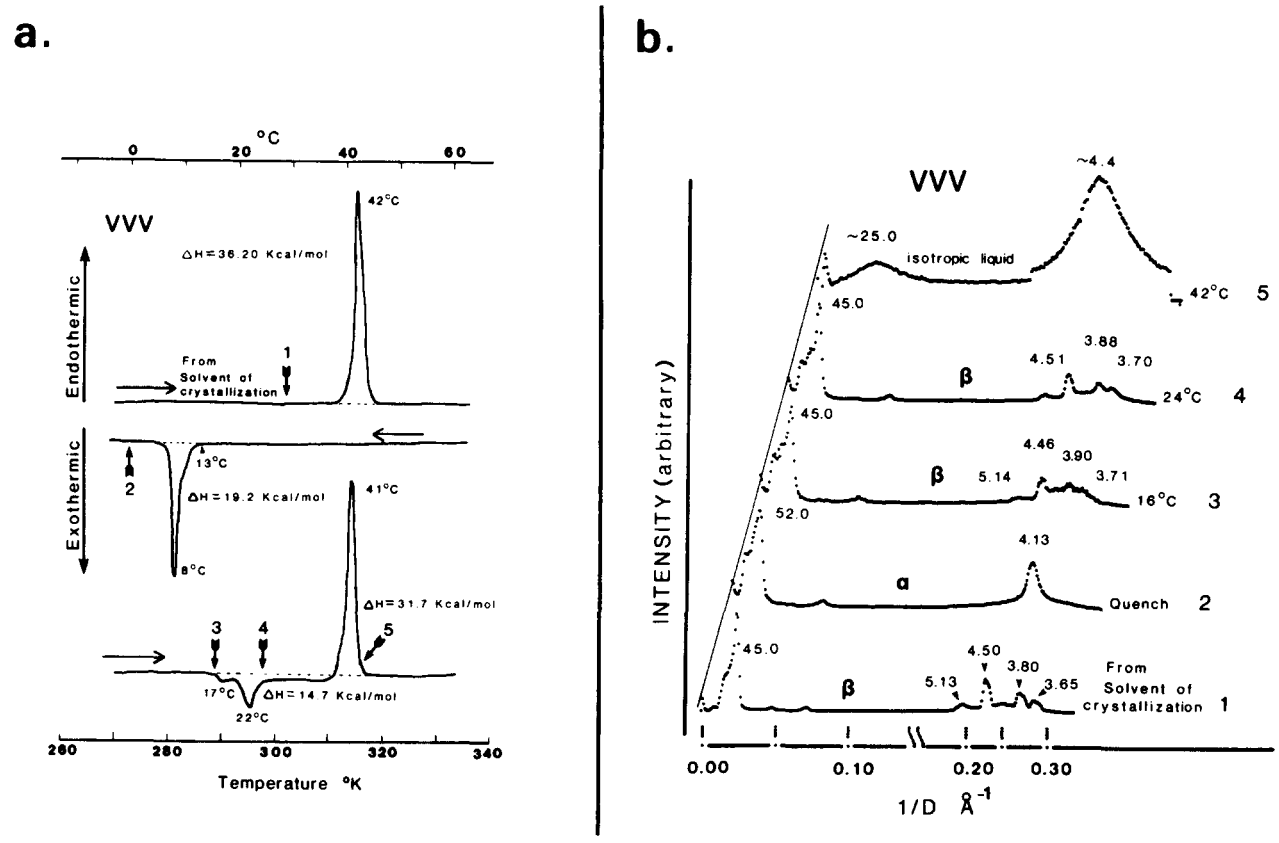

Fig. 2. a: Differential scanning calorimetry scans of trivaccinoylglycerol. b: X-ray diffraction patterns of trivaccinoylglycerol at different temperatures. Refer to Fig. 1 legend for details.

ity of 65 Å (**Fig. 3b1**). Upon cooling, the isotropic liquid began to crystallize at −7°C (peak at −10°C) with 10.8 Kcal/mol enthalpy of formation. The quenched X-ray diffraction sample (at −7°C) gave the diffraction pattern of an α-phase (strong diffraction corresponding to 4.05 Å) and a long spacing periodicity of 52 Å (Fig. 3b2). On heating, the α-phase melted at −6°C and immediately recrystallized with an exothermic peak transition at −4°C. The crystallization continued with another small exothermic peak at 9.5°C and was followed by a final endothermic transition at 25°C to an isotropic liquid with an enthalpy of melting of 27 Kcal/mol. The X-ray diffraction pattern of the α-phase on standing at −7°C for ~20 min transformed into a diffraction pattern typical of a β'-phase (two short D-spacings, 4.12 and 3.85 Å) with a long spacing periodicity of 45 Å (Fig. 3b3). Upon raising the sample temperature to 0°C, the strong diffraction peak corresponding to 4.46 Å typical of the β-phase started to appear (Fig. 3b4). At 10°C, long spacings of both 45 Å and 65 Å were present. The intensity of the 45 Å peak decreased as the 65 Å peak increased with increasing time and temperature. The transition of X-ray diffraction long spacing from 45 Å to 65 Å on raising the temperature from 0 to 23°C is shown in Figs. 3b4–7. The change in the long spacing periodicity was accompanied by an increase in intensity of the diffraction corresponding to 4.5 Å. The multiple short spacings obtained at 23°C (Fig. 3b7) were virtually identical to the β-phase diffraction pattern obtained for the phase from solvent of crystallization (Fig. 3b1).

OEO

As this compound is a liquid at room temperature, it was cooled to −53°C and then immediately reheated to 0°C and incubated at this temperature for an hour to allow it to crystallize into a stable polymorphic form. On reheating after the incubation, an endothermic transition to an isotropic liquid observed at 9°C with an enthalpy of transition of 28.5 Kcal/mol (**Fig. 4a**). The same thermal behavior was observed after prolonged incubation (~15 hr) at lower temperatures (−8°C) demonstrating that the stable polymorph had been obtained. The X-ray diffraction pattern of the sample incubated at 0°C for 1 hr showed a strong wide-angle diffraction (D-spacing 4.53 Å, Fig. 4b1) indicating a β-phase with a molecular long axis periodicity of 66 Å. The isotropic liquid on cooling began to crystallize at −28°C (peak at −39°C) with an enthalpy of formation of 12.75 Kcal/mol. The X-ray diffraction sample on quenching from isotropic liquid in dry ice-acetone

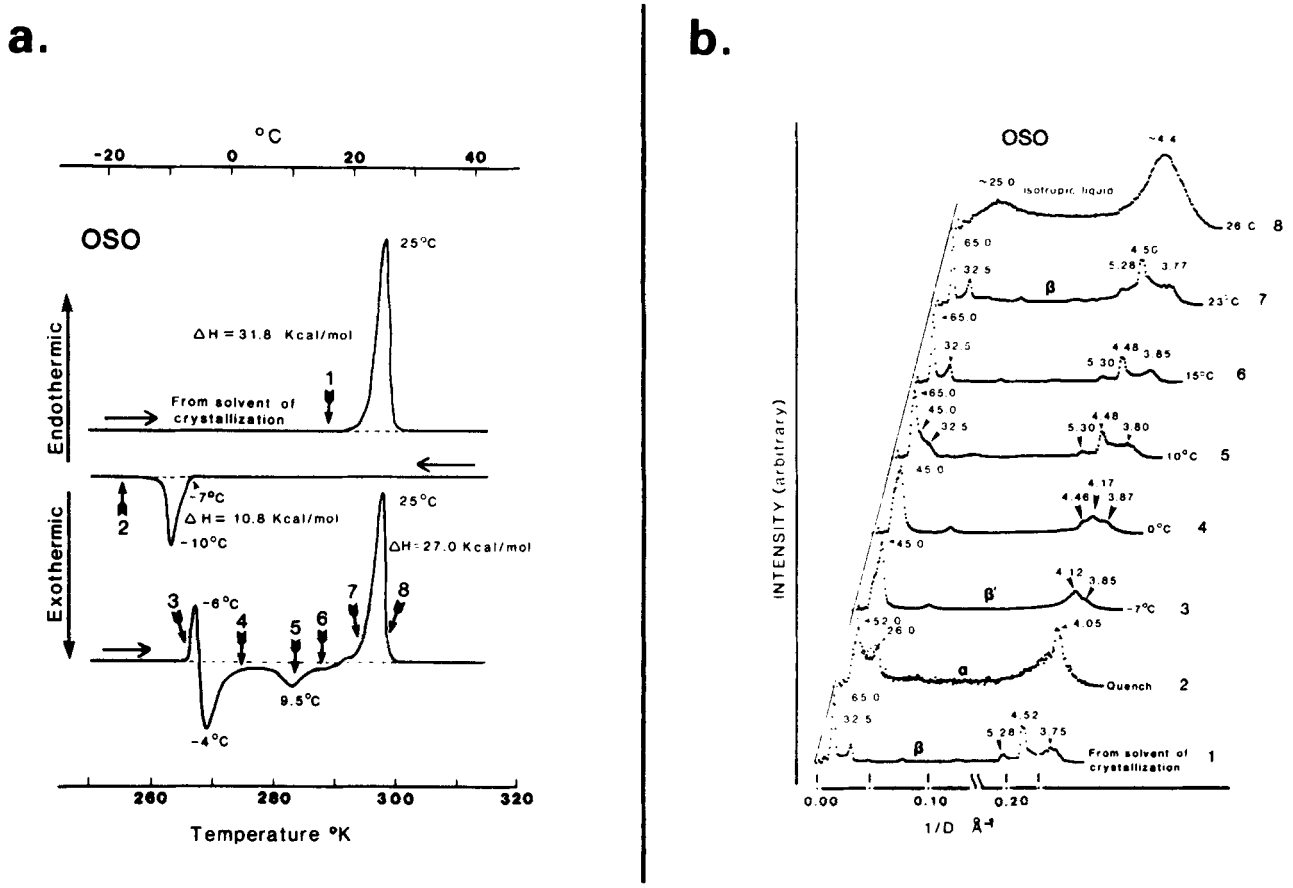

Fig. 3. a: Differential scanning calorimetry of 1,3-dioleoyl-2-stearoylglycerol. b: X-ray diffraction patterns of 1,3-dioleoyl-2-stearoyl glycerol at different temperatures. Refer to Fig. 1 legend for details.

mixture gave a diffraction pattern typical of a β'-phase with 4.22 and 3.87 Å diffraction peaks and 45 Å long spacing (Fig. 4b2). The DSC reheating scan of the polymorph crystallized on cooling showed a broad low temperature exothermic transition indicating further crystallization. The polymorph thus crystallized melted at −0.5°C with 16.5 Kcal/mol enthalpy of transition. This endotherm was immediately followed by a broad exotherm and then a small endotherm indicating melting to an isotropic liquid at 8°C. The X-ray diffraction pattern of the quenched sample did not change until −5°C. However, further increase in temperature produced multiple short spacings that were accompanied by a new long spacing at 66 Å (Fig. 4b3). As the temperature was raised, the 4.5 Å band intensified and other wide-angle diffraction lines became sharper (Fig. 4b4, 4b5). In the long spacing region, the intensity of the diffraction corresponding to 66 Å increased as the 45 Å peak decreased. The diffraction pattern at −1°C (Fig. 4b5) was similar to the diffraction of the β-phase obtained after the incubation at 0°C (Fig. 4b1). The β-phase X-ray diffraction intensity decreased on further increase in temperature from 8 to 10°C, and at 11°C the X-ray diffraction pattern was that of an isotropic liquid (Fig. 4b6).

OVO

The stable polymorph was obtained by preincubating the sample at −10°C for 40 hr. It is characterized by β short spacings and a 65 Å long spacing. The DSC heating scan after the incubation showed an endothermic transition to an isotropic liquid with the peak transition temperature of 4°C and an enthalpy of transformation of 22.9 Kcal/mol. The X-ray diffraction pattern of this phase after 40 hr incubation at −10°C showed multiple short spacings with an intense wide-angle diffraction (D-spacing 4.42 Å) typical of a β-phase with a 65 Å long spacing (**Fig. 5b1**). This phase melted to an isotropic liquid on increasing the sample temperature to 5°C (Fig. 5b2). When the length of incubation of the sample at −10°C was decreased from 40 to 16 hr, the DSC heating scan showed a broad endotherm with two peak transition temperatures at 1.5°C and 4°C with a total enthalpy of 21.1 Kcal/mol. The X-ray diffraction pattern of the sample taken at −10°C corresponding to the same length of incubation (16 hr) at the same temperature showed multiple diffractions (D spacings 5.3, 4.42, and 3.89 Å) with a relatively intense diffraction at the D value 3.89 Å (Fig. 5b3). The long spacing region showed

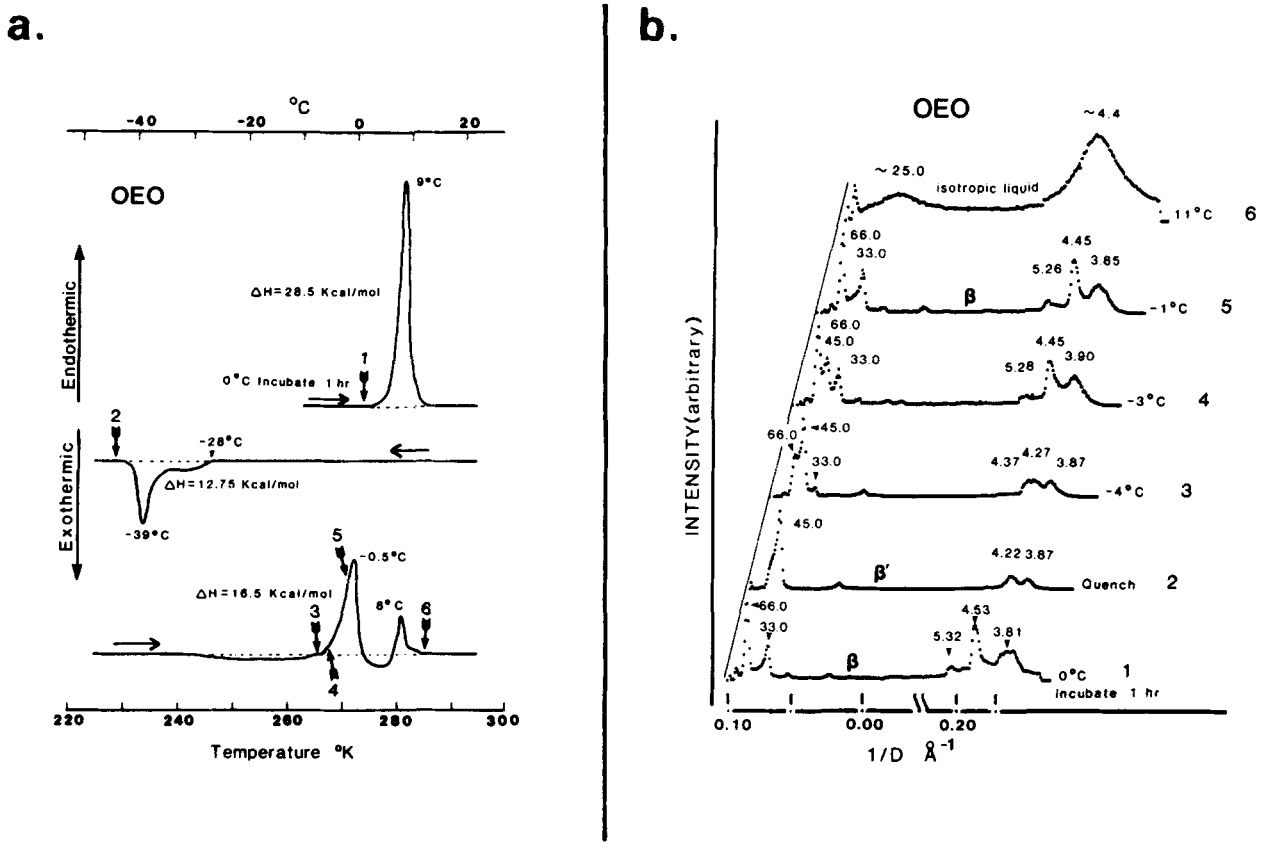

Fig. 4. a: Differential scanning calorimetry of 1,3-dioleoyl-2-elaidoylglycerol. b: X-ray diffraction patterns of 1,3-dioleoyl-2-elaidoylglycerol at different temperatures. Refer to Fig. 1 legend for details.

two first order diffraction peaks with D values of 65 Å and 45 Å (Fig. 5b3). On increasing the temperature of the sample, the diffraction corresponding to 65 Å increased in intensity and that at 45 Å decreased and eventually disappeared at 3°C. In the short spacing region, on increasing the sample temperature, the relative intensity of the diffraction D-spacing 4.42 Å increased in comparison to that of 3.9 Å peak (Fig. 5b5). Further increase of temperature produced the diffraction pattern typical of an isotropic liquid at 5°C (Fig. 5b6). The compound, on cooling from the isotropic liquid, crystallized at −40.5°C with an enthalpy of formation of 14.5 Kcal/mol. On immediate reheating, the phase obtained after crystallization showed a broad exotherm followed by an endotherm with a peak transition temperature of 1°C and an enthalpy of 18.1 Kcal/mol. To obtain the phase crystallized on cooling the isotropic liquid, the X-ray diffraction sample was quenched in dry ice–acetone. The diffraction pattern of the sample taken at −10°C, immediately after quenching, is shown in Fig. 5b7. The short spacing region showed two peaks (D spacing 4.2 and 3.85 Å) typical of a β'-phase diffraction with a long spacing periodicity of 45 Å. On further increasing the temperature, this diffraction intensity started to decrease. At 2°C the diffraction pattern was that of an isotropic liquid (Fig. 5b9). The physical properties of the diacid TGs OSO, OEO, and OVO are presented in **Table 2.**

DISCUSSION

There are several reports in the literature on the polymorphism of monoacid TGs (1). In general, the α-phase is the lowest melting polymorph with hexagonal chain packing with nonspecific chain–chain interactions. β'-phase is intermediate in melting between that of α- and β-phases and the acyl chains pack with specific chain–chain interactions, with the alternate acyl chains packing in planes perpendicular to each other along their long axes. The common subcell packing of β'-phase is orthorhombic perpendicular ($0\perp$) (1, 18). The β-phase is the most stable crystal form with the highest melting temperature of the three polymorphic states and can usually be obtained from solvent of crystallization. In this phase the methylene chains are packed parallel to each other and the common subcell packing is triclinic parallel ($T\parallel$) (1).

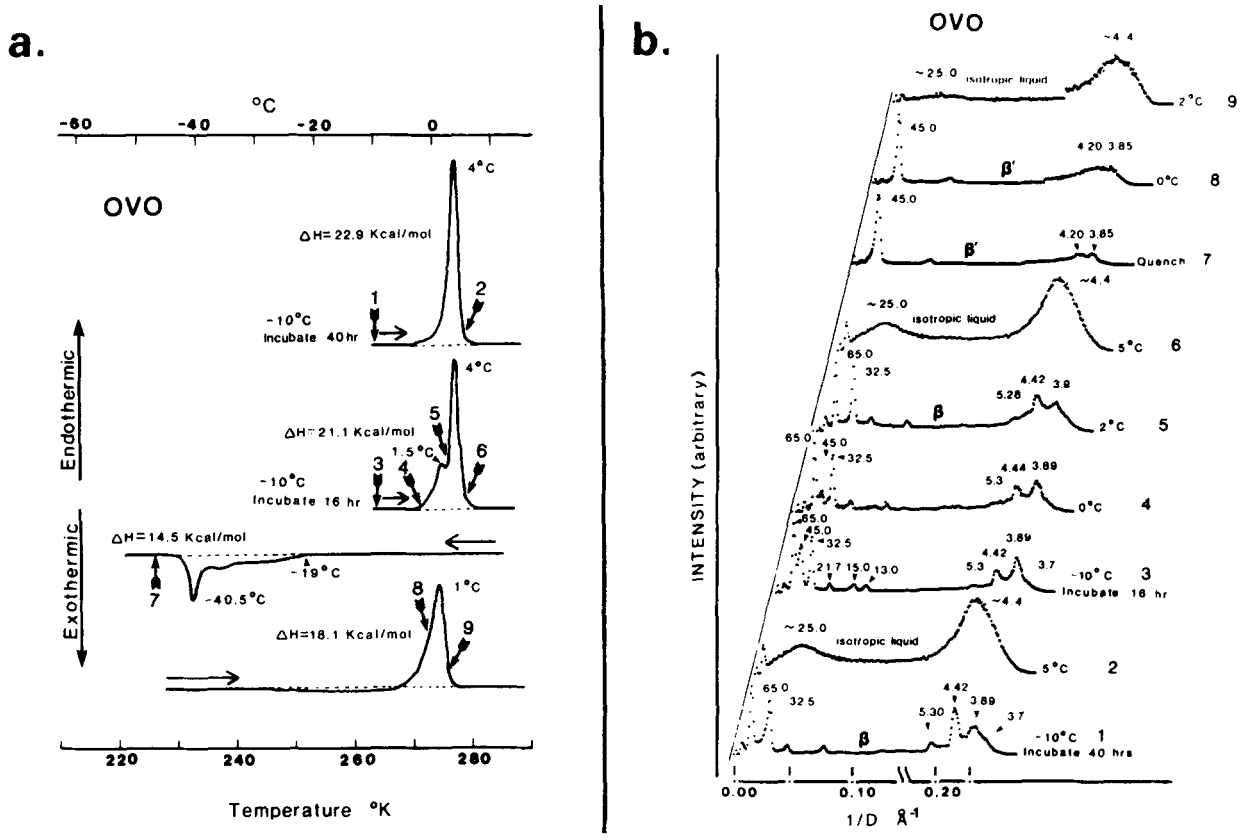

Fig. 5. a: Differential scanning calorimetry of 1,3-dioleoyl-2-vaccinoylglycerol. b: X-ray diffraction patterns of 1,3-dioleoyl-2-vaccinoylglycerol at different temperatures. Refer to Fig. 1 legend for details.

The physical characteristics of the monoacid TGs studied in this work have been compared to those from previous reports (4, 11–15) are given in Table 1. The thermotropic phase behaviors of these compounds typified by SSS and EEE are schematically represented in **Fig. 6a and 6b,** respectively. The melting temperatures of the α- and β-forms of the single acid TGs decreased in the sequence SSS, EEE, OOO, with EEE and VVV showing very similar transition temperatures. A similar trend was observed in the enthalpy of melting of β-form to the isotropic liquid of these compounds. The thermal and X-ray diffraction data of VVV and EEE were very similar except that the VVV did not show any melting of the α-phase before it was transformed to the β-phase. During the transformation of α- to β-phase (Figs. 1b and 2b), no evidence of β' short spacings was apparent in these two compounds. Therefore, β'-phase does not appear to be even a short-term intermediate between α- and β-phases.

TABLE 2. Physical properties of diacid triglycerides

	Melting Point				ΔH			Long Spacings		
Triglyceride	α	β'	β	T_c[a]	α or β'[b]	β'	β	α	β'	β
		°C		°C		*Kcal/mol*			Å	
OSO	-6	[c]	25	-7	10.8	[c]	31.8	52	45	65
OEO	[d]	-0.5	9	-28	12.7	16.5	28.5	[d]	45	66
OVO	[d]	1	4	-19	14.5	18.1	22.9	[d]	45	65

[a]Temperature of onset of crystallization.
[b]Enthalpy of formation on cooling (crystallization to either α or β' as appropriate).
[c]β' → β transition is exothermic.
[d]Not observed.

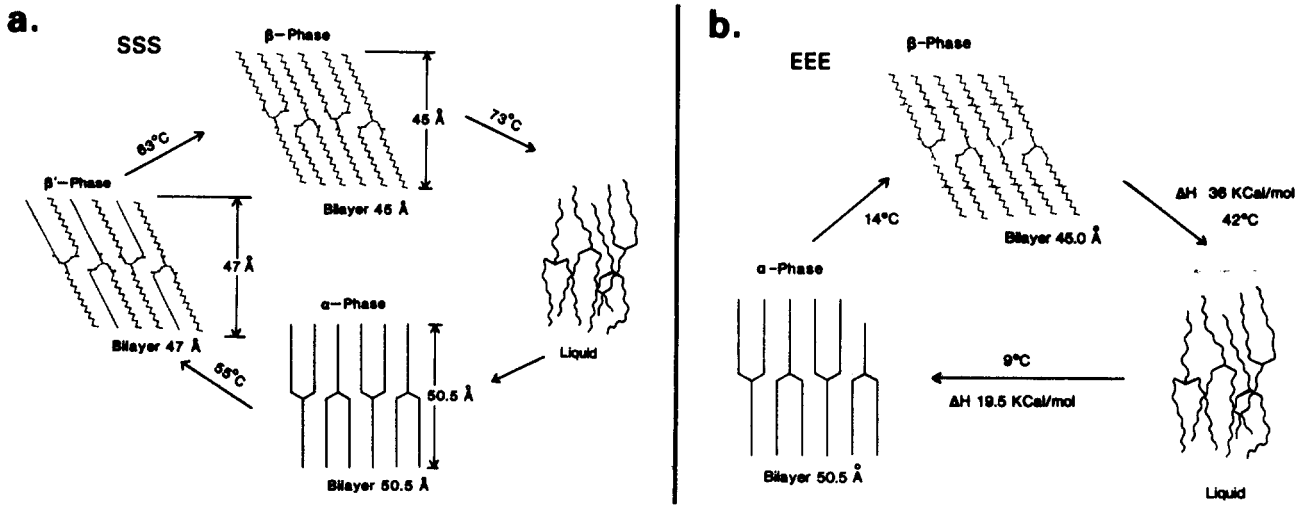

Fig. 6. a: Schematic representation of the phase behavior of tristearin showing the bilayer structure in α, β', and β-phases (from refs. 4 and 11). This phase behavior is similar to other saturated monoacid TGs (ref. 1). b: Schematic representation of phase behavior of trielaidoylglycerol showing α and β-phases. Phase behaviors of trivaccinoylglycerol is also similar. The bilayer periodicity is indicated on each schematic structure and the transition temperatures are shown on the arrows connecting the schematic structures.

In EEE and VVV, the melting temperatures and enthalpies of melting of the β-phase obtained from solvent of crystallization were higher than the β-phase obtained after melting (Figs. 1a and 2a). This might be partly attributed to the difference in size and perfection of the β-crystals obtained from solvent of crystallization and from the melt. In both compounds, the α to β transformation shows a continuous exothermic transition until the beginning of the β-melting, indicating that the β-phase formation was not completed before it started to melt. This was confirmed by incubation of the sample for 15 hr at a few degrees below the melting temperature of the β-phase. After this incubation, the high melting temperature and enthalpy were recovered.

The long spacings of the monoacid TGs in α-, β'-, and β-forms varied between 43 and 52 Å indicating a bilayer structure for an 18-carbon triglyceride, assuming that these compounds pack in a manner similar to that of tricaprin (19) and trilaurin (20). The longer bilayer periodicity of the α-phase indicates that the tilt angle of the methylene chains from the basal methyl plane is nearer to the right angle than that in the β- or β'-phases.

In the diacid TGs (OSO, OEO, and OVO), the β-phase is the stable polymorphic form. The melting temperatures and the enthalpy of melting of this phase to the isotropic liquid is in the order OSO > OEO > OVO. The repetition periodicity of ~65 Å suggests that the β-phase of these compounds packs in a trilayered structure where the middle layer, containing the 2-stearoyl, elaidoyl, or vaccinoyl chains, is flanked on either side by 1- and 3-oleoyl chains. In these three compounds the differences in the melting temperatures and enthalpies of the β-phase can be interpreted in terms of the changes in the packing of the middle layer. The general phase behavior of these compounds as typified by OSO is given in **Fig. 7.**

Quenching OSO from the isotropic liquid produced a bilayered α-phase, whereas the OEO and OVO produced a β'-phase. The enthalpy of first crystallization of OEO and OVO was similar and greater than that of OSO, suggesting that the phase obtained on quenching OSO is different from that of OEO and OVO. However, we cannot rule out the possibility of the existence of α-phase in OEO and OVO as the onset of first crystallization (Figs. 4a, 5a) indicated a shoulder. The α-phase of OSO melted before crystallizing into a β'-phase. The β' to β transformation of this compound (Fig. 3a) is a net exothermic transition without melting of the β'-phase, indicating that the β-phase is relatively much more stable than the β'-phase. However, there is a difference of 5 Kcal/mol in enthalpy of melting of the β-form obtained on crystallization and the β-phase formed after melting. This difference could be due to the size and perfection of the crystals and incomplete transformation of β'-phase into β, before the melting of β-phase. The X-ray diffraction experiments (3b–5b) on OSO, OEO, and OVO showed that the changes in the short spacing region (from β'-phase short spacings to β-phase short spacings) and the changes in the long spacing periodicity from 45 Å to 65 Å occurred concurrently. This indicates that the β' to β transformation and conversion of a bilayer to a trilayer organization are synchronous processes.

The DSC behavior of OEO (Fig. 4a) shows that the β'-phase melts and then crystallizes partially into β-phase before it completely melts into an isotropic liquid. The

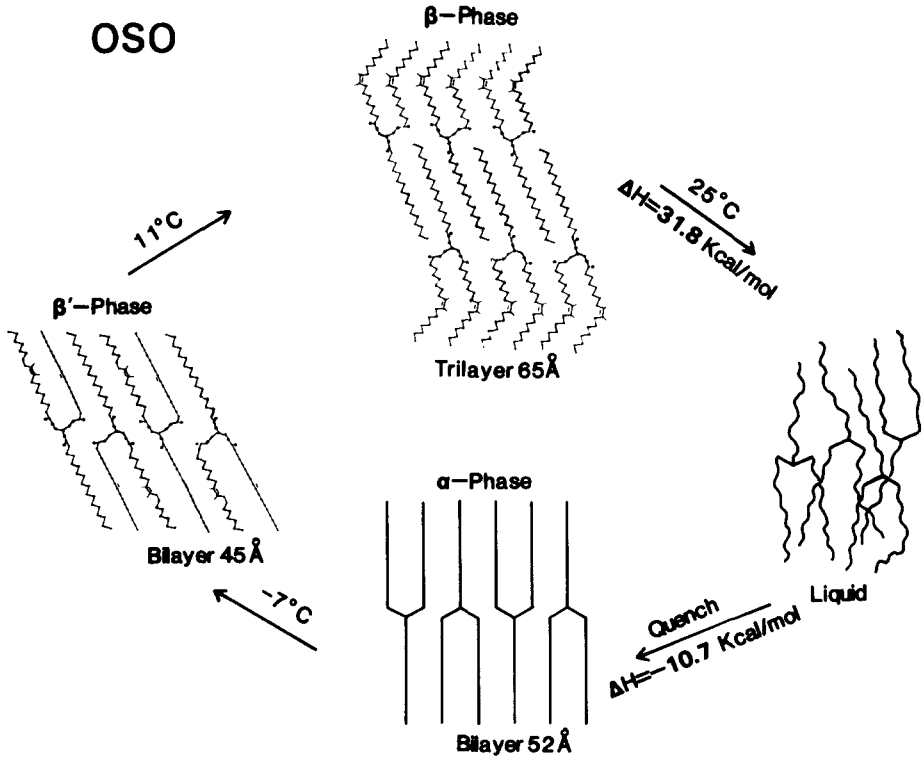

Fig. 7. Schematic representation of the phase behavior of 1,3-dioleoyl-2-stearoylglycerol showing a bilayer packing in α and β'-phases and a trilayer structure for β phase. The transition temperatures are shown on the arrows. The enthalpy of melting of β phase and the enthalpy of formation of α phase are given.

complete conversion of this compound into β-phase requires a short incubation (~1 hr) near the melting temperature of β'-phase. Thus the β'-phase is relatively stable; however, the β-phase is the most stable polymorph.

The DSC behavior of OVO (Fig. 5a) shows that the β'-phase melts into an isotropic liquid without any crystallization into β-phase. To crystallize this compound into the β-phase requires a prolonged incubation (~40 hr) near the melting temperature of the β'-phase. When the incubation time was relatively short (~16 hr), both β'- and β-phases were obtained (Fig. 5b3). This fact, combined with relatively small differences in the enthalpy (4.8 Kcal/mol) and melting temperature (3°C) of the β'- and β-phases, suggests that the stability of β'-phase is comparable to that of β-phase.

The long spacing periodicities of the β'- and β-phases of all the diacid TGs indicate a bilayer and trilayer organization, respectively. The relative stability of a bilayered β'-phase versus trilayered β-phase of these diacid TGs can be interpreted in the following way. In the bilayered β'-phase, the oleoyl chains do not segregate from the stearoyl, elaidoyl, or vaccinoyl chains. Therefore, stability of this phase depends upon how well each of these saturated or *trans*-unsaturated acyl chains pack with the oleoyl chains, compared to the packing of the segregated middle layer of saturated or *trans*-unsaturated acyl chains in the trilayered β-phase.

In summary, the monoacid TGs, SSS, OOO, EEE, and VVV pack in a bilayered (D = 43–52 Å) structure in all the polymorphic states. The *trans*-unsaturated TGs, EEE and VVV, do not form a β'-phase. The mixed diacid TGs, OSO, OEO, and OVO, pack in a trilayered structure (D = ~65 Å) in the stable β-phase, where the saturated or *trans*-unsaturated 2-acyl chains segregate from the oleoyl chains and form a middle layer flanked on either side by two different layers of 1,3-oleoyl chains. The β'-phase of these compounds packs in a bilayered structure. The glycerol backbone configuration in both bilayered β'-phase and trilayered β-phase of these compounds is similar to that of the monoacid TGs. Therefore, it can be concluded that the driving force to form a trilayered structure in the β-phase of these compounds is the inability of the saturated or *trans*-unsaturated acyl chains to pack along with the bent (*cis*) oleoyl chains.

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REFERENCES

1. Small, D. M. 1986. Glycerides. *In* The Physical Chemistry of Lipids, From Alkanes to Phospholipids. Plenum Press, New York. Chapter 10, 345-372.
2. Morley, N. H., A. Kuksis, and D. Buchena. 1974. Hydrolysis of synthetic triacylglycerols by pancreatic and lipoprotein lipase. *Lipids.* **9:** 481-488.
3. Redgrave, T., and D. R. Kodali. 1985. Effects of triacylglycerol structure on the metabolism of chylomicrons and emulsion models of chylomicrons. 7th International Symposium on Atherosclerosis, Melbourne, Australia.
4. Lutton, E. S., and A. J. Fehl. 1969. The polymorphism of odd and even saturated single acid triglycerides C_8-C_{22}. *Lipids.* **5:** 90-99.
5. Chapman, D. 1962. The polymorphism of glycerides. *Chem. Rev.* **62:** 433-456.
6. Larsson, K. 1964. Solid state behavior of glycerides. *Ark. Kemi.* **23:** 35-56.
7. Larsson, K. 1972. Molecular arrangement in glycerides. *Fette Seifen Anstrichm.* **74:** 136-142.
8. DeJong, S., and T. C. Van Soest. 1978. Crystal structures and melting points of saturated triglycerides in the β-2 phase. *Acta Crystallogr.* **B34:** 1570-1583.
9. Kodali, D. R., D. Atkinson, T. G. Redgrave, and D. M. Small. 1984. Synthesis and polymorphism of 1,2-dipalmitoyl-3-acyl-*sn*-glycerols. *J. Am. Oil Chem. Soc.* **61:** 1078-1084.
10. Fahey, D. A., D. M. Small, D. R. Kodali, D. Atkinson, and T. G. Redgrave. 1985. Structure and polymorphism of 1,2-dioleoyl-3-acyl-*sn*-glycerols. Three- and six-layered structures. *Biochemistry.* **24:** 3757-3764.
11. Hagemann, J. W., W. H. Tallent, and K. E. Kolb. 1972. Differential scanning calorimetry of single acid triglycerides: Effect of chain length and unsaturation. *J. Am. Oil Chem. Soc.* **49:** 118-123.
12. Carter, M. G. R., and T. Malkin. 1947. X-ray and thermal examination of the glycerides. VIII. Glycerides of crucic, brassidic, oleic and elaidic acids. *J. Chem. Soc.* 554-558.
13. Ferguson, R. H., and E. S. Lutton. 1947. The polymorphism of triolein. *J. Am. Chem. Soc.* **69:** 1445-1448.
14. Hagemann, J. W., W. H. Tallent, J. A. Brave, I. A. Ismail, and F. D. Gunstone. 1975. Polymorphism in single-acid triglycerides of positional and geometric isomers of octadecenoic acid. *J. Am. Oil. Chem. Soc.* **52:** 204-207.
15. Filer, L. J., Jr., S. S. Sidhu, B. F. Daubert, and H. L. Longenecker. 1946. X-ray investigation of glycerides. III. Diffraction analysis of symmetrical monooleoyl-disaturated triglycerides. *J. Am. Chem. Soc.* **68:** 167-171.
16. Bentley, P. H., and W. McCrea. 1970. An efficient synthesis of symmetrical 1,3-diglycerides. *J. Org. Chem.* **35:** 2082-2083.
17. Franks, A. 1958. Some developments and applications of microfocus X-ray diffraction techniques. *Br. J. Appl. Phys.* **9:** 349-352.
18. Abrahamsson, S., B. Dahlen, H. Lofgren, and I. Pascher. 1978. Lateral packing of hydrocarbon chains. *Prog. Chem. Fats Other Lipids.* **16:** 125-143.
19. Jensen, L. H., and A. J. Mabis. 1966. Refinement of the structure of β-tricaprin. *Acta Crystallogr.* **21:** 770-781.
20. Larsson, K. 1964. The crystal structure of the β-form of trilaurin. *Arkiv. Kemi.* **23:** 1-15.